Application No.: 10/748,094

## IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently amended) A process for manufacture of long circulating non-pegylated liposomes comprising:

dissolving one or more phospholipids and one or more sterols in a solvent or mixture of solvents;

forming a lipid film by evaporating a solvent from a lipid solution comprising one or more phospholipids, a sterol and a solvent; and

hydrating the <u>phospholipids and sterols</u>[[ film]] with an aqueous hydration media <del>to form</del> non-pegylated liposomes;

removing the solvent or mixture of solvents before or after hydrating the lipids; wherein the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present in the lipid solution form non-pegylated liposomes; and wherein the aqueous hydration media comprises ammonium sulfate and sucrose; and wherein the one or more phospholipids is a saturated phosphatidylcholine selected from the group consisting of distearoyl phosphatidylcholine (DSPC), dipalmitoyl phosphatidylcholine (DPPC), hydrogenated soya phosphatidylcholine (HSPC) and derivatives thereof; and

wherein the one or more phospholipids exhibits a phase transition temperature of between 50 and 65°C; and

wherein the non-pegylated liposomes have a blood circulation half life of at least 25 times longer than conventional non-liposomal formulations when tested in Swiss albino mice at equivalent doses.

wherein the forming and the hydrating are performed without the addition of polyethylene glycol (PEG).

- 2. (Original) The process of claim 1 wherein the amount of aqueous hydration media used is 30 ml for each mmole of phospholipid in the lipid solution.
- 3. (Original) The process of manufacture of non-pegylated liposomes of claim 1 further

Application No.: 10/748,094

comprising loading the liposomes with a therapeutic or diagnostic agent.

- 4. (Original) The process of claim 3, wherein the therapeutic agent is an antineoplastic agent.
- 5. (Original) The process of claim 4, wherein the antineoplastic agent is selected from the group consisting of Doxorubicin hydrochloride, Daunorubicin hydrochloride, and Epirubicin hydrochloride.
- 6. (Original) The process of claim 5, wherein the antineoplastic agent is Doxorubicin hydrochloride.
- 7. (Original) The process of claim 1, wherein the molar ratio of phospholipid to sterol is from about 1:0.1-1:2.
- 8. (Previously Presented) The process of claim 7, wherein the molar ratio of phospholipid to sterol is from about 1:0.7.
- 9. (Previously Canceled).
- 10. (Previously Presented) The process of claim 1, wherein the concentration of ammonium sulfate in aqueous hydration media is not less than 125 mmoles/liter.
- 11. (Currently Canceled).
- 12. (Currently Amended) The process of claim [[11]] 1, wherein the phospholipid has a minimum of sixteen carbons fatty acid chain.
- 13. (Currently Canceled).

Application No.: 10/748,094

14. (Currently Amended) The process of claim [[13]]1, wherein the phospholipid is distearoyl phosphatidylcholine (DSPC) and wherein the sterol is cholesterol.

- 15. (Original) The process of claim 1, wherein the non-pegylated liposomes are successively extruded through series of filters having pore sizes from 0.4 µm to 0.05 µm for sizing.
- 16. (Original) A liposome manufactured by the process of claim 1.
- 17. (Original) The liposome of claim 16, wherein the phospholipid comprises distearoyl phosphatidylcholine (DSPC) and the sterol comprises cholesterol.
- 18. (Original) The liposome of claim 16, wherein the non-pegylated liposome further comprises a therapeutic or diagnostic agent.
- 19. (Original) The liposome of claim 18, wherein said therapeutic agent comprises an antineoplastic agent.
- 20. (Original) The liposome of claim 19, wherein the antineoplastic agent is selected from the group consisting of Doxorubicin hydrochloride, Daunorubicin hydrochloride, and Epirubicin hydrochloride.
- 21. (Original) The liposome of claim 20, wherein the antineoplastic agent is Doxorubicin hydrochloride.
- 22. (Original) The liposome of claim 16, wherein the average size of liposome is 0.06 μm to 0.16 μm in diameter.
- 23.-60. (Previously Canceled).

Daftary et al.

Docket No. B235 1010.1

Application No.: 10/748,094

- 61. (Currently Canceled).
- 62. (Currently Amended) The process of claim 1, further comprising removing the solvent before after hydrating the lipid film; wherein the amount of the aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present in the lipid solution; sizing the non-pegylated liposomes to about 0.06 µm to form a liposomal composition; removing extra-liposomal hydration salt from the liposomal composition using <u>a</u> sucrose-histidine buffer solution to form non-pegylated [[size]]<u>sized</u> liposomes.